THE EFFECT OF CENTRIFUGING ON THE POLARITY OF AN ALGA, GRIFFITHSIA BORNETIANA

VICTOR SCHECHTER

(From the Department of Biology, College of the City of New York, and the Marine Biological Laboratory, Woods Hole, Mass.)

Introduction

The nature of differentiation and particularly the causes of the axial differentiations which define the polarity of an organism, are problems which have been attacked from many different angles. Experimental procedures for a long time centered about the concept of specific organ-forming substances. At first the simple expedient of overturning fragments was utilized to observe the effect of heavier and lighter substances upon regeneration through the action of gravity. This method, employed chiefly with plant material, showed that although orientive adjustments occurred, there was usually little effect upon the actual point of origin of new roots and shoots (e.g. Vöchting, 1885, 1906; Loeb, 1924). Exceptional cases, perhaps most clearly illustrated in the lower plants (Noll, 1900; Wulff, 1910) were explained, often by the authors themselves, as due to the stimulation of preformed centers by accumulated indifferent nutrients.

At Naples, in 1901, Morgan attempted to analyze the effect of gravity on the regeneration of Antennularia through the use of a "rotating wheel." The centrifuge method, initiated by Lyon in 1906 and adopted by many others,[1] may have developed from this simple device. As with gravity, however, it was shown clearly that centrifugal displacement of visible granules is ineffective in altering the normal pattern of growth, except of course in the event of injury. Lillie (1909) therefore concluded that polarity is a property of the ground substance; and Conklin (1931) decided that the effect of extremely high speeds (upon the development of ascidian eggs) is due to the displacement of specific areas in the cytoplasm.

The case which I wish to present in this paper has several points of interest. It is the only one I know of in which a new axis of polarity, involving normal organ formation, is set up by centrifugal force. The

[1] Morgan and Lyon, 1907; McClendon, 1909; F. R. Lillie, 1909; Morgan, 1908, 1909, and 1910; Morgan and Spooner, 1909; Boveri, 1910; Conklin, 1931 and previously.

results are obtained at surprisingly low speeds, which renders improbable the displacement of cytoplasmic areas. The facts are of interest, also, because of the light which they may be construed to throw upon the doctrine of "specific stuffs."

Material and Method

During the course of an investigation on the effect of direct electric current upon regeneration in the alga, *Griffithsia bornetiana* (Schechter, 1934), electrophoresis of chromatophores to the position where rhizoids arose was frequently observed. Preliminary experiments with the centrifuge to determine whether there was any causal relationship between these two phenomena gave negative results, but indicated an interesting effect upon the shoots. The present report is concerned with this effect. The experimental work was done chiefly during the summer of 1934, and the substance of the results presented at the Marine Biological Laboratory at the seminar of August 7, 1934. I wish to acknowledge gratefully helpful conferences with Dr. L. G. Barth.

Freshly collected tufts of the alga were cut into suitably sized fragments and centrifuged continuously for about 24 hours with a force of approximately 150 × gravity. Stratification of the cell contents began in about an hour and after 24 hours the chromatophores were accumulated as a dense cap of material at the centrifugal pole, sharply marked off from the rest of the cell which had become quite transparent. The fragments were then allowed to develop in Syracuse dishes of sea water until new shoots appeared. An ocular micrometer was used for measuring and sketches were made with the aid of a camera lucida.

In the early experiments there was much damage to the material, arising toward the end of the period of centrifuging. By avoiding overcrowding and carrying out the experiments at 19° C. (7–9° below room temperature during the hot weeks of 1934), injury was much reduced and often almost entirely avoided. Under carefully controlled conditions one batch of material was centrifuged continuously for a period of four days with a force of 61 × gravity. Shoots and rhizoids were regenerated.

After the usual amount of centrifuging (about 150 × gravity for 24 hours) it required a week or more before redistribution of the cell contents restored the normal appearance. This unusual duration of stratification may be of significance with respect to the effect upon polarity.

Results

Out of 18 experiments the material died in three, probably due to high temperature, and in 12 of the remaining 15 there was more or less determination of shoot origin in each, as described below:

When the cell apexes were oriented outward from the axis of rotation shoots always appeared in normal positions (Fig. 1, sketches 12, 13, 14). On the other hand, as shown in sketches 1, 2, 6, 8 and Photographs 4 through 12, shoots often arose from the base when the cell

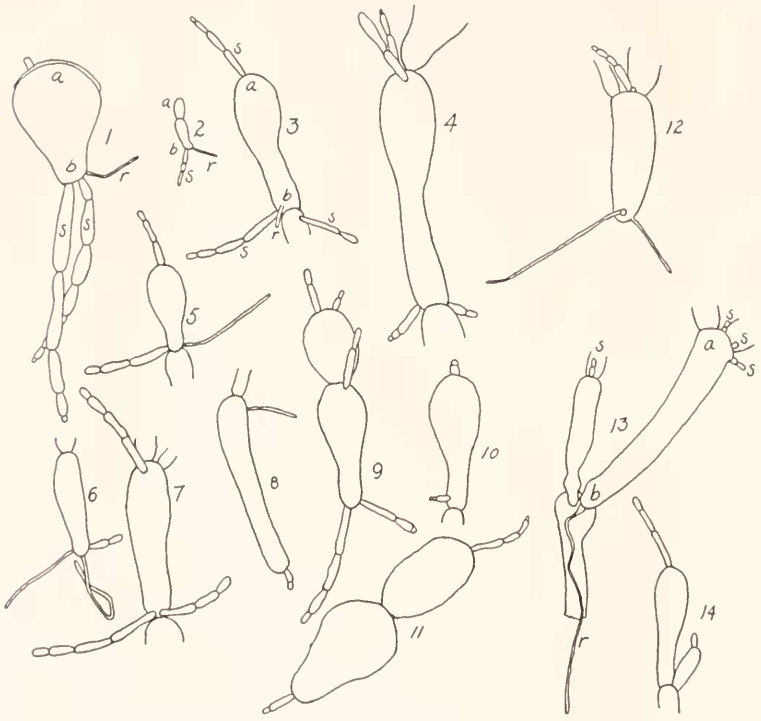
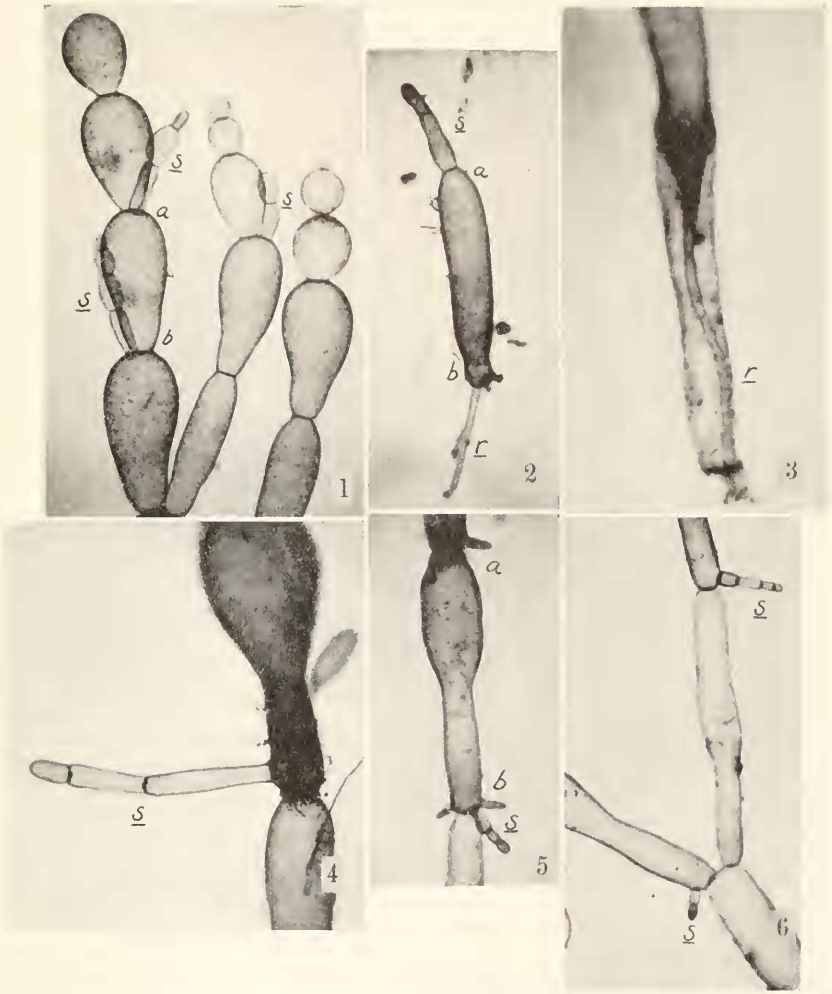


Fig. 1. Camera lucida sketches of experimental material.

Sketches 1–11 of material centrifuged basally. Induced basal, and some shoots in normal position, are shown on the same or different cells.

Sketches 12–14 of material centrifuged apically. The normal polarity was retained. (*a*—cell apex, *b*—base, *s*—new shoot, *r*—rhizoid.)

bases were centrifugally oriented. However, basal shoot formation did not necessarily exclude proliferation from the apexes of the same cells (sketches 3–5, 7, 9–11). It is to be noted that all of the shoots were quite normal in appearance. For comparison several young shoots on control material are shown in Photograph 1. Photographs 2 and 3 are of basally centrifuged cells in which the normal polarity nevertheless remained unaltered.



EXPLANATION OF PLATES

Plate I

Plates I and II contain photographs of control (Photograph 1) and of experimental material centrifuged basally.

1. Control showing two-, three- and four-celled new shoots in normal apical position.

2. A cell, centrifuged basally, in which the original polarity was retained.

3. The base of a cell with material accumulated during centrifugation. Original polarity was retained in this case as shown by rhizoid in normal position.

4, 5, 6. Baso-lateral shoots following centrifugation. The cells were still part of the original filament, where they occupied a position about midway between apex and base of the plant.

An inspection of the angle of induced shoots upon isolated cells and upon those cells still part of a filament, reveals an interesting situation. The new shoots came off laterally (Photographs 4, 5, 6) when upon cells forming part of a filament. On free cells they usually arose directly from the base (Photographs 8, 10, 11, 12); or there was occasionally a shoot in each position. Photograph 7 shows a male apical cell partially dislodged from the chain with both a lateral and an almost directly basal shoot. In Photograph 9, where the adjoining cell is dead, the basal shoot has grown directly through the dead region. These observations indicate a tendency toward a rather local effect directly in line with the axis of centrifuging.

Unlike shoot formation, rhizoid origin from centrifuged cells was not materially affected. In fact rhizoids often appeared on the cell base together with induced shoots (Photograph 11). In Photograph 12 a new shoot, by forming a rhizoid on its own axis, has completely established independent polarity.

The series of photographs demonstrates also that shoot-forming polarity may be reversed in cells anywhere along the axis of the alga, from the extreme apex to the extreme base.

Data concerned with the frequency with which reversals occur is based mainly upon four experiments. In cells oriented so that heavier materials were thrown toward the apex 229 apical shoots were formed. When oriented in the opposite direction a roughly equivalent number of cells produced 214 apical and 121 basal shoots. The centrifugal forces in these experiments were 100 to 220 $\times$ gravity, an average of 160 $\times$.

It seems interesting that the new shoots were generally smaller in the experiments than in the controls. In one case, for example, 14 typical shoots upon basally centrifuged cells averaged 2.6 cells in number (range 2–5) and 0.75 mm. in length. Where the heavier substances were thrown into the cell apexes 17 shoots, varying from 1 to 6 cells, averaged 3.3 cells and 1.17 mm. in length. Eleven typical shoots upon control material were 3.0 mm. in average length and consisted of from 2 to 9 cells, an average of 6.5. Perhaps correlated with their smaller size is the general observation that new shoots occurred more frequently on centrifuged cells. No exact data on this point are available at present.

These measurements, besides showing a difference between shoots on centrifuged and non-centrifuged cells, also bring out the fact that orientation of the cells during centrifugation affected the size of new shoots. A summation of the data of several experiments indicates that the difference is a general one. One hundred and four apical shoots (unaffected in origin) upon basally centrifuged material averaged 2.9 cells and 0.9 mm. in length; whereas 41 basal shoots consisted of 2.54

cells and were 0.78 mm. long. Also supporting are measurements made in one experiment upon 15 basally centrifuged cells each of which formed both apical and basal shoots, as in sketches 5 and 10, Fig. 1. The average length of the former was 0.85 mm. and of the latter 0.76 mm.

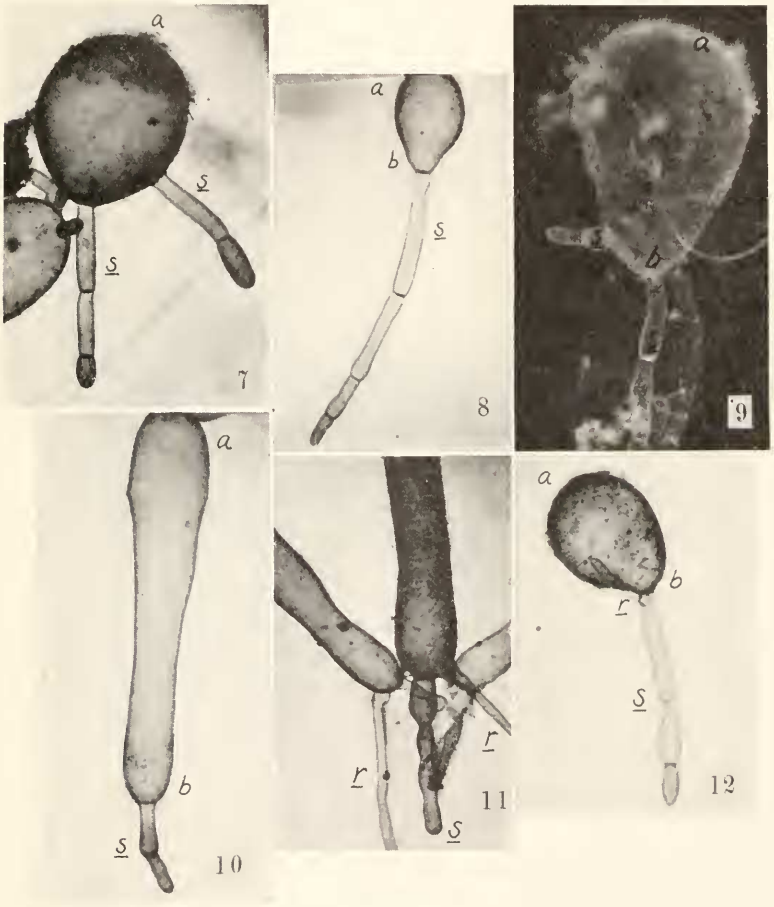


PLATE II

7. An apical cell partially dislodged from the filament. One new shoot formed laterally, one almost directly basally.

8. An isolated apical cell. New shoot directly basal.

9. An apical cell held to the filament by a dead adjoining cell. A basolateral shoot and a basal shoot were formed.

10. A basal cell. Basal shoot on the free basal end.

11. Basal cells. Induced basal shoot and normal basal rhizoids are both present.

12. A basal shoot from an apical cell. The basal cell of the new shoot formed a rhizoid normal to the polarity of the induced shoot.

In all of the instances cited above the basal (induced) shoots were smaller than the apical. If we may assume a fairly regular rate of growth, then the size of shoots is an indication of the time at which they began to form and the difference in size should be a measure of the amount of centrifuging required to induce basal shoot formation.

Discussion

In view of numerous unsuccessful attempts to control polarity by centrifuging and the absence of reports in this direction incidental to the large amount of other work involving the same technique, the results presented in this paper appear to comprise the only case in which a normal determinative effect upon development has been obtained. It is therefore of particular importance to investigate the underlying mechanism of centrifuge action in this material. To be sure, the syncytial nature of the *Griffithsia* cell (see Lewis, 1909) offers a different type of biological system than do animal eggs and embryos, upon which practically all other work involving centrifugation has been done. It may be that some feature of organization peculiar to a syncytium makes the results possible. But in nature *Griffithsia* shoots are formed on the cell apexes regardless of orientation with respect to gravity. We would have to concede to the centrifuge, therefore, a greater efficiency and perhaps a higher specificity in the separation of cellular elements than can be attributed to gravity.

The effect obtained by centrifuging *Griffithsia* also seems, in other respects, somehow unrelated to the action of gravity. Loeb (1894) found that hydranths of Antennularia were regenerated from the upper ends of overturned stems. With plants, where it has been possible to obtain responses other than those of orientation, results have been similar. In all of these cases the new growths formed at the upper ends were those normally found in that position in nature. Quite on the contrary with *Griffithsia;* after centrifuging the new shoots were produced where the heavier materials had been thrown, a position synonymous with the lower end of the organism in the field of gravity; one normally associated with rhizoid formation.

The possibility therefore arises that the effect is due, not to the movement of shoot-forming substances, but to a stimulation set up by an unusual concentration of rather non-specific materials. The simultaneous appearance of shoots on both poles of some of the cells also weighs against the existence of a definite shoot-forming substance, necessarily limited in amount, and is in accord with the above hypothesis. An analogy can perhaps be drawn with activation of unfertilized eggs by various chemical and physical agencies.

May I, in concluding, take this opportunity to acknowledge my indebtedness for help and stimulation to Dr. Charles F. Hunt, whose understanding interest extended from medicine to other fields of science, and whose recent death has removed a rare and valued friend.

Summary

Normal shoots appear upon *Griffithsia* cells at the point where heavier substances are concentrated by prolonged low speed centrifuging. In this way reversal of polarity may be produced anywhere along the plant axis. The possibility is suggested that the centrifuged substances are not directly determinative but act by stimulation.

LITERATURE CITED

Boveri, Th., 1910. Über die Teilung Centrifugierter Eier von Ascaris megalocephala. *Arch. Entw.-mech.*, **30**: 101.

Conklin, E. G., 1931. The Development of Centrifuged Eggs of Ascidians. *Jour. Exper. Zool.*, **60**: 1.

Lewis, I. F., 1909. Life History of Griffithsia bornetiana. *Ann. Bot.*, **23**: 639.

Lillie, F. R., 1909. Polarity and Bilaterality of the Annelid Egg. Experiments with centrifugal force. *Biol. Bull.*, **16**: 54.

Loeb, J., 1894. On Some Facts and Principles of Physiological Morphology. Biological Lectures at the Marine Biological Laboratory in 1893. Ginn and Co., Boston.

Loeb, J., 1924. Regeneration from a Physicochemical Viewpoint. New York.

Lyon, E. P., 1906. Some Results of Centrifugalizing the Eggs of Arbacia. *Am. Jour. Physiol.*, **15**: xxi (Proc. Am. Physiol. Soc.).

McClendon, J. F., 1909. Cytological and Chemical Studies of Centrifuged Frog Eggs. *Arch. Entw.-mech.*, **27**: 247.

Morgan, T. H., 1901. The Factors that Determine Regeneration in Antennularia. *Biol. Bull.*, **2**: 301.

Morgan, T. H., 1908. The Effects of a Centrifugal Force on the Eggs of Cumingia. *Science*, **27**: 446.

Morgan, T. H., 1909. The Effects Produced by Centrifuging Eggs Before and During Development. *Anat. Rec.*, **3**: 155.

Morgan, T. H., 1910. Cytological Studies of Centrifuged Eggs. *Jour. Exper. Zool.*, **9**: 593.

Morgan, T. H., and E. P. Lyon, 1907. The Relation of the Substances of the Egg, Separated by a Strong Centrifugal Force, to the Location of the Embryo. *Arch. Entw.-mech.*, **24**: 147.

Morgan, T. H., and G. B. Spooner, 1909. The Polarity of the Centrifuged Egg. *Arch. Entw.-mech.*, **28**: 104.

Noll, F., 1900. Über die Umkehrungsversuche mit Bryopsis. *Ber. d. Deut. Bot. Gesell.*, **18**: 444.

Schechter, V., 1934. Electrical Control of Rhizoid Formation in the Red Alga, Griffithsia bornetiana. *Jour. Gen. Physiol.*, **18**: 1.

Vöchting, H., 1885. Über die Regeneration der Marchantieen. *Jahrb. f. wissensch. Botanik*, **16**: 367.

Vöchting, H., 1906. Über Regeneration und Polarität bei höheron Pflanzen. *Bot. Zeitung*, **64**: 101.

Wulff, E., 1910. Über Heteromorphose bei Dasycladus clavæformis. *Ber. d. Deut. Bot. Gesell.*, **28**: 264.